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Bee venom ameliorates methyl mercury-induced reproductive impairment in male Sprague Dawley rats

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ABSTRACT

Background: Exposure to organic mercury leads to a range of serious fertility-related complications. **Purpose:** The present study aimed to evaluate the restorative potential of bee venom against testicular injury induced by the administration of methyl mercury chloride (MMC) to rats. **Materials and methods:** Rats were randomly divided into four groups as follow; Group I rats were gavaged PBS (NaCl 0.9%), Group II rats were subcutaneously injected with BV (0.5 mg/kg BW), Group III rats were gavaged MMC (6.7 mg/kg BW) and Group rats were coadministered MMC followed by BV with the same doses and routes of groups II and III. Sexual efficiency parameters were estimated in semen. Serum levels of testosterone, luteinizing hormone (LH) and Follicle stimulating (FSH) hormone were measured using ELISA technique. The activities of Sorbitol dehydrogenase (SDH), lactate dehydrogenase (LDH) and gamma glutamyl transferase (GGT) were determined in testicular tissues. **Results:** The administration of BV to MMC-intoxicated rats led to a noticed improvement of sperms quality and count compared to control. Moreover, a significant increase of testicular hormones and enzymes was observed in MMC-intoxicated rats received BV compared to control. **Conclusion:** Our findings suggest that BV has a repairing capacity against MMC-induced testicular damage in male rats.

1. Introduction:

Mercury (Hg) is a global, highly toxic, and persistent pollutant in the environment. Hg is used in several products and released from combustion processes. The moment that Hg exists in the environment, it can be changed to the organic form methyl mercury (MeHg) and transferred up the food chain, where it accumulates in fish and marine mammals [**1, 2**]. MeHg is an environmental contaminant worldwide [**3].** The primary environmental route of MeHg exposure is from dietary sources to aquatic systems [**1].**

Environmental exposure to MeHg may occur chronically at low levels, with fish consumption being the main

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source for human intoxication [**4**]. Exposure to MeHg, due to industrial, agricultural, and mining activity, still raises concerns about risks to human health [**5**]. It is well known that MeHg produces reproductive dysfunctions including hypospermia, loss of libido and erectile dysfunction [**6].** MeHg altered testicular morphology and produced damage to spermatogenic processes that led to apoptosis of germ cells **[7].** In addition to decreases in testosterone serum levels **[7,8,9]**and infertility [10], MeHg either increased or decreased reproductive organs weights **[7],** diminished sperm count and motility as well as damaged sperm morphology in monkeys and rodents **[11]**

The majority of the experimental studies conducted using MeHg to examine effects on reproductive toxicity involved the administration of various doses and varying treatment regimens, and focused mainly on testis **[7,8,12,13]**

MeHg has been shown to affect male reproductive function. Hg inhibits the activity of Leydig cell enzymes involved in steroidogenesis and membrane function, resulting in impaired production of sex steroids, such as testosterone **[9,14].** Furthermore, impairment of the production of testosterone can disturb male reproduction by impairing spermatogenesis, among other effects **[15].** Previous research has shown that exposure to Hg led to a significant decline in the number of spermatogonia, spermatocytes and spermatids in testes of rats **[16].** Shino Homma-Takeda et al. have shown that MeHg impairs male rat spermatogenesis, resulting in germ cell deletion via cell- and stage-specific apoptosis **[7].**

Honeybees produce different bee products including, honey, bee venom, royal jelly, pollen, propolis, and bee wax. These products containing many biologically active constituents such as minerals, vitamins, and polyphenols **[17],** which have functioned as preventive and therapeutic apitherapy in the last four decades**.** Currently, there are several studies reporting the possible protective and therapeutic effects of such bee products in health, especially male infertility **[18,19].** BV is produced by the female worker bees. It is usually delivered directly from a bee sting. Bee venom therapy (BVT) or apitherapy is a natural therapy used in alleviating pain and inflammation by either live bee stings or purified and diluted BV injections **[20].** It has been practiced by the ancient Greeks, Chinese, and Egyptians to treat many diseases such as osteoarthritis, rheumatoid arthritis, fatigue, and skin diseases **[21,22].** Furthermore, some substances extracted from bee venom can be included in domestic animal diets as antimicrobial agents to enhance productive performance and health status **[23].**

BV comprises of peptides such as melittin and apamin, mast cell degranulating (MCD) peptide, adolapin, histamine, tertiapin, secapin, melittin F, and cardiopep as well as enzymes such as phospholipase A2 (PLA2), phospholipase B (PLB), hyaluronidase (cytotoxicity), dopamine, serotonin, phosphatase, and α-glucosidase (nontoxic) **[22,24,25].** Bee venom has a variety of pharmaceutical properties including analgesic **[26],** anticancer **[27],** antibacterial **[28],** antifungal **[29]**, antiviral **[30]** neuroprotective **[31]**, and the treatment of many skin conditions **[32].** Few studies have been reported on the effects of BV on the testicular damage; Egyptian bee venom at doses of 0.1, 0.2, and 0.3 mg/rabbit twice weekly administered over 20 weeks showed increases in TAC, GST, GSH, testosterone spermatogenesis, and fertility **[23].** In a related study carried out in mice treated with Iraqi bee sting, it provided protection and the maintenance of some sexual efficiency parameters via its ability to release cortisol that inhibits Sertoli cells from releasing activin-B, which normally stimulates spermatogonia to induce mitosis to form spermatocytes **[33].**

Recently, the adverse health effects of MeHg and protective effects against its toxicity have become an active area of research. Up to date, there are no established remedies to rescue Hg

toxicity **[34]**. Chelation therapies have been established for use in Hg intoxication **[35],** however, chelating agents alone are not satisfactory to treat signs of poisoning. It is probable that combining with other alternative therapeutic agents, like antioxidants remedy, might alleviate Hg-induced toxicity. Combination remedy targeted at counteracting OS and chelation may have a synergistic and consequently superior outcome. Several studies have revealed that the antioxidants or selenium could reduce MeHg-induced toxicity either in vivo or in vitro **[36,37].**

To the best of our knowledge, this is the first study aimed to explore the potential role of Bee venom against MMC-induced reproductive disturbance.

2.Materials and methods:

2.1. Chemicals

The lyophilized pure Egyptian honey bee venom (BV) (*Apis mellifera lamarckii*) was obtained from Department of Bee Research, Plant Protection Institute, Ministry of Agriculture, Egypt, kept desiccated at 4ºC when needed reconstituted by the addition of sterile phosphate buffer saline (PBS; pH 7.2). Methylmercury chloride (CH3HgCl), product number (442534-5G-A), CAS-No: 115-09-3 99% purity, molecular weight: 350.59) was obtained from Sigma-Aldrich Chemical (St. Louis, MO, USA). All the chemicals of analytical grade, kits and enzymes used in the present study were obtained from established firms such as Sigma.

2.2. Experimental animals

Forty-eight male albino rats weighing 150- 200 gm were used in the current study. Rats were purchased from the laboratory animal's farm, Faculty of Veterinary Medicine, Zagazig University. The animals were accommodated to the laboratory conditions for two weeks before being experimented, allowed to access freely to water and feed throughout the acclimatization and experimental periods.

After a period of two weeks of acclimatization, the rats were weighed and randomly divided into four groups, Group I (control): In which the rats were gavaged PBS (NaCl 0.9%) (1 ml/rat), Group II Bee venom exposed group (BV): The rats were subcutaneously injected with BV at a dose of 0.5 mg/kg body weight , Group III: methyl mercuric chloride exposed group (MeHg): The rats were gavaged MeHgCl at a dose of 6.7 mg/kg ,Group IV (MeHg+BV): the rats were co administered MeHg followed by BV with the same doses and routes of group 3 and 2.

All animal handling and experimental protocol were performed in accordance with the general guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals in scientific investigations, evaluated, and approved by the Zagazig University Research Center Institutional Animal Care and Use Committee (IACUC) under number ZU-IACUC/1/F/18/2020. All rats were weighed before and after the experiment, and at the end of the experimental period the behavioral tests were performed, blood samples, semen picture and tissue samples were collected then biochemical parameters were measured.

2.3. Samples collection:

The serum samples were preserved at -20 °C until used for determination of hormone levels **[38]**. The cauda epididymis of one testis was excised and received in a sterilized Petri dish containing 2 ml warm normal saline of 37ºC, then it was macerated by sterilized scissor to obtain the epididymal contents in a suspension that was handled as the semen **[39].**

At the end of the treatment period rats were sacrificed. 30 mg of testicular tissue were snap frozen in liquid nitrogen and stored in - 80 °C. Part of one testis from each experimental animal was homogenized by a WiseTis HG-15D homogenizer (Daihan Scientific Co., Seoul, Korea) estimation of testicular enzymes activities (GGT, LDH, and SDH).

2.4. Determination of biochemical

parameters

Testosterone, Luteinizing hormone (LH), Follicle stimulating hormone (FSH), sorbitol dehydrogenase (SDH), lactate dehydrogenase (LDH) and gamma glutamyl transferase (GGT) were estimated in testicular tissues using commercial ELISA kits and the procedures were followed according to the manufacturer instructions.

2.5. Statistical analysis:

The data of this current study were statistically analyzed by using computer program of SPSS/PC+2001. With statistical method of the one way ANOVA was estimated, followed by the Duncan's multiple range test **[40]**. The results are presented as the means plus or minus criterion error. Minimum level of the indication was set at $p < 0.05$.

3. Results:

3.1. Effect of (MeHgCl) and/or BV and their combinations on Body and testicular weight, semen evaluation parameters, serum sex hormones levels, and testicular enzymes of male rats

There were no significant differences in the IBW between different experimental groups. BV non-significantly increased final body weight (FBW) but significantly increased testes weight as compared with the Control group. MeHgCl significantly decreased FBW and testes weight in the MeHg intoxicated as compared with the C group. However, BV administration in the MeHgCl +BV treated group significantly restored the decreased FBW and testes weight compared with the C group.

BV significantly increased sperm motility, sperm count, testosterone, FSH, SDH, LDH, GGT and non-significantly increased LH and GSI as compared with the Control group. MeHgCl significantly decreased sperm motility, sperm count, testosterone, FSH, SDH, LDH, GGT, LH and GSI in the MeHgCl intoxicated group as compared with the Control group. However, BV administration in the MeHgCl +BV treated group significantly restored the decreased sperm motility, sperm count, testosterone, FSH, SDH, LDH, GGT, LH and GSI as compared with the C group.

BV non-significantly decreased sperm abnormalities as compared with the C group. MeHgCl significantly increased sperm abnormalities in the MeHgCl intoxicated group as compared with the C group. However, BV administration in the MeHgCl +BV treated group significantly restored the increased sperm abnormalities compared with the C group.

4. Discussion:

Methylmercury (MeHg) is a widespread environmental pollutant causes a serious hazard to testicular development and spermatogenesis. However, molecular mechanisms underlying male reproductive toxicity induced by MeHg remain elusive **[41].**

In the present study, MeHg significantly decreased body weight (29.23% decrease) and BV administration in the MeHg+BV treated group significantly restored the decreased FBW compared with the C group. MeHg results were in agreement with **[42],** who proposed a relationship between high MeHg doses and caloric restriction .The reduction of body weight might be due to anorexia which accounted for reduction of food consumption.

Reproductive organ weights are indicators of reproductive toxicity of a chemical **[43]**. Our results revealed that BV significantly increased testes weight as compared with the C group. MeHg significantly decreased testes weight in the MeHg intoxicated group. However, BV administration in the MeHg+BV treated group significantly restored the decreased testes weight to comparing with the C group.

The decrease in testes weight could be the most sensitive parameter representing the male gonadal toxicity. It is well known that sufficient amount of androgen supplementation is important to sustain the structural and

functional integrity of male reproductive system. Thus, the lowered serum testosterone levels in our treated rats may be attributed to the reduction in testis weight **[44**].

The differences among the various studies are possibly due to the use of different MeHg concentrations, treatment periods, animal model and/or administration routes (e.g. subcutaneous injection or oral through gavage) **[9].**

BV administration in the MeHg+BV treated group non-significantly restored the decreased GSI to (1.59 % increase) compared with the C group. The significant increase in the weight of testes at MeHg+BV treated group can be explained due to congestion and edema demonstrated in histopathological examination which makes the organ heavier than normal **[45]**.

Al-Sayigh et al. 2012 **[33]** found that BV can affect the sexual efficiency by increasing testes weight and improving semen characteristics quality. These effects could be attributed to pituitary gland stimulation to release Adrenocorticotropic hormone that causes release of the sex hormones such as testosterone in blood circulation which have significant effects on spermatogenesis and fertility **[46].**

Concerning semen quality, the present study showed that MeHg significantly reduced the sperm cell count and sperm motility where the sperm abnormalities were significantly increased. Methylmercury has been shown to affect male reproductive function. **[7]** have shown that MeHg impairs male rat spermatogenesis, resulting in germ cell deletion via cell- and stage-specific apoptosis. Normal spermatogenesis is highly dependent on natural differentiation and development of germ cell ; conversely, GC dysfunction leads to oligoasthenospermia, or even infertility**[41].**

Mercury inhibits the activity of Leydig cell enzymes involved in steroidogenesis and membrane function, resulting in impaired production of sex steroids, such as testosterone **[9]**. Furthermore, impairment of the production of testosterone can disturb male reproduction by impairing spermatogenesis, among other effects. Previous research has shown that exposure to mercury led to a significant decline in the number of spermatogonia, spermatocytes and spermatids in testes of rats **[16].**

The decline in the concentration of testosterone following MeHg treatment indicates harmful changes in the Leydig interstitial cells of testes, which are responsible for testosterone biosynthesis and secretion **[47]** Furthermore, the reduction in sperm count could be attributed to the decline in the concentration of testosterone through induction of Leydig cell damage that disrupts testosterone synthesis. Adequate levels of testosterone are needed to maintain normal spermatogenesis **[47]** which results in normal sperm count, while decreased sperm motility could be due to DNA damage **[48],** testicular oxidative stress, and increase in phospholipids peroxidation **[49].**

Sperm morphology is under genetic control **[50]** involving several autosomal and Y-specific genes. Increases in abnormal sperm morphology are important indicators of genetic damage. Abnormal sperm morphology is usually caused by DNA mutations and chromosomal aberrations that occur during the compaction of DNA in the sperm head **[51]** that are undergoing spermatozoon differentiation and maturation during the process of spermatogenesis. Reduction in sperm parameters is consistent with the histopathological analysis of the testicular sections of the testes of mice treated with MeHg that showed significant harmful changes that occurred in the seminiferous tubules, including the detachment of Sertoli cells, sloughing of spermatogonia, increased interstitial space, degenerative changes of the Leydig interstitial cells, and irregular organization of seminiferous tubules with distortion of the testicular structure **[52].**

BV significantly increased sperm motility by (13.0% increase) and increased sperm count by (16.44% increase) as compared with the C group in addition to decreased sperm abnormalities. BV significantly increased sperm count and decreased abnormal sperm ratio stimulate proliferation of leydig cells as feedback mechanism. Furthermore, the sperm motility, concentration, alive sperm and fertility percentage were significantly higher in BV group. These results indicate that BV has a beneficial impact on male spermatogenesis, and this impact was BV dose-dependent manner **[23].**

These results are disagreement with **[52,53]** who provided evidence for the damaging effect of bee venom on histoarchitecture of rat testes, low semen quality and adverse testicular histological changes in male mice treated with bee venom. The repeated administration of bee venom by sting or injection to adult male mice results in harmful reproductive effects as evidenced by the decrease in serum testosterone levels, reduction in sperm parameters, especially normal sperm morphology, and histological damage to the testes. and conclude that bee venom is a testicular toxic agent **[18].**

Hormones play an important part in defining the optimal conditions for reproductive life to start**.** Testosterone as the main sex hormone in males plays an important role in sexual differentiation, maturation, development of male sexual organs and maintenance of spermatogenesis which regulated by the pituitary FSH and LH **[54]**.

In the present study, there was a significant reduction in serum testosterone levels in rats treated with MeHg. Based upon the endpoints determined, it is not possible to explain the reason for this observed reduction **[11]**

Maines and Mayer[55] proposed that the decrease in testosterone levels after MeHg exposure is due to functional damage to Leydig cells consequently resulting in changes in the synthesis of 17-hydroxylase, a P-450 cytochrome dependent key enzyme involved in the hormone biosynthesis. It is well-known that MeHg adversely alters male steroidogenesis**[9].**

Oxidative stress is one of the main causes of male infertility **[56]**. Sperm are extremely sensitive to oxidative stress **[57]**, the elevated levels of which can significantly reduce sperm motility and viability affect sperm–egg interaction **[58]** and cause irreparable damage to the sperm **[59]**. It can impact spermatogenesis and other aspects of reproductive health, since when in high concentrations; ROS are related to sperm destruction, DNA damage, organelle breakdown and cell death **[60].**

Several studies have illustrated that oxidative stress acts as a master mechanism of MeHg toxicity. The mechanisms of MeHg-induced oxidative stress involve the excessive accumulation of ROS and the inhibition of antioxidant enzymes **[4].** However, a detailed investigation of the role of oxidative stress in MeHg reproductive toxicity remains unknown. In this study, we have demonstrated that MeHg is able to inhibit the activity of the antioxidant enzymes, including GST and GPx in addition to increase NO and MDA levels.

Concerning the MMC-induced OS, the obtained results showed that MMC significantly reduced GSH, SOD, CAT, GPx, and GST levels in the testis of the treated rats. Moreover, MMC induced a significant increase in the NO, MDA, PCO, and 8OH2dG of MMC exposed rats. Several reports have shown that neurotoxicity induced by MeHg is related to augmented levels of ROS **[61].**

Regarding the observed pathological alterations induced by MeHg, these changes may be attributed to oxidation and reduction reactions that convert it to inorganic Hg releasing ROS **[62].** MeHg releases ROS causes severe damage to cells by triggering the series of lipid peroxidation of the cell membrane. Additionally, MeHg has been proven as a high fat-soluble, is

toxic to the CNS which has a high-fat content **[63].** Hg can inhibit thiol group-containing enzymes, interacts with membrane proteins, and can substitute zinc in certain zinc-activated enzymes or replace metallothionein-bound Zn, Cu, and Cd. The ability of Hg to interact with phospholipids and specific enzyme systems may help explain the cell degeneration, apoptosis, and necrosis, and overall toxicity observed.

Regarding the modulating effect of BV against MMC-induced OS, the obtained results confirmed the in vivo antioxidant properties of BV via regulating the levels of OS-related indices of MMC exposed rats. The obtained results are co-operating with those obtained by **[64,65];** who observed that BV could protect against OS and this might be attributed to the antioxidant potentials of different BV constituents such as PLA2, melittin, and apamin owing to their ability to prevent the LPO and enhancing the SOD enzyme activity **[66].**

BV non-significantly increased GST by (0.07% increase) , significantly increased GPX by (11.74% increase) and decreased MDA by (7.74% decrease) as compared with the C group. These results were in agreement with the results of **[32]** on broiler chickens. They concluded that bee venom added in broiler diet can increase antioxidant activity. This means that BV has antioxidant characteristics that maintain the semen quality against high-temperature conditions damages in summer months by reducing oxidative stress. BV has pharmacological antioxidant activities, thus, it can protect against cellular damage, and this effect may be associated with reduced oxidative damage to lipids, proteins and DNA.

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Table 1. Effect of methyl mercury chloride (MeHgCl) and/or Bee venom (BV) administration on body weight, testes weight, gonadosomatic index, epididymal semen picture, serum sex hormones, testicular enzymes of Sprague Dawley male rats. (n=10) (Means ± SD).

Groups Parameter	Control	BV	MeHgCl	MeHgCl +BV
Initial B.W (g)	175± 3.16 ^{a}	178.33 ± 6.83 ^{a}	178.33± 6.05 ^{a}	179.16± 3.76 ^{a}
Final B.W (g)	230.75± 6.38 ^{a}	235.48± 6.68 ^{a}	163.31± 15.62 ^{b}	177.66± 13.66 ^{b}
Testes weight (g)	1.46± 0.10 ^{b}	1.68± 0.17 ^{a}	0.65± 0.12 ^{d}	1.15± 0.05 ^{c}
Gonadosomatic index	0.64± 0.05 ^{a}	0.71± 0.08 ^{a}	0.40± 0.10 ^{b}	0.65± 0.04 ^{a}
Sperm count ($x10^{6}$/mL)	97.33± 4.32 ^{a}	113.33± 7.52 ^{a}	59.66± 15.42 ^{b}	73.16± 10.98 ^{b}
Sperm motility (%)	83.33± 2.58 ^{b}	94.16± 4.91 ^{a}	38.33± 6.05 ^{d}	68.33± 7.52 ^{c}
Sperm abnormalities (%)	11.50± 1.22 ^{bc}	5.83 ± 1.47 ^{c}	30.33± 10.67 ^{a}	18.83± 6.17 ^{b}
Testosterone (ng/mL)	3.64± 0.32 ^{b}	6.92± 1.45 ^{a}	1.27± 0.074 ^{c}	2.31± 0.40 ^{c}
LH (mIU/ml)	10.22± 0.05 ^{a}	11.23± 0.013 ^{a}	5.84± 1.26 ^{c}	8.18± 0.41 ^{b}
FSH (ng/mL)	2.33± 0.39 ^{ab}	2.96± 0.54 ^{a}	1.32± 0.41 ^{c}	1.79± 0.49 ^{bc}
SDH (ng/ml)	9.01± 1.51 ^{b}	13.08± 1.35 ^{a}	3.28± 0.45 ^{c}	5.53± 1.98 ^{c}
LDH (U/L)	136.00± 5.58 ^{b}	168.66± 9.47 ^{a}	93.83± 4.26 ^{d}	114.16± 16.38 ^{c}
GGT (U/L)	68.00± 6.26 ^{b}	87.33± 6.71 ^{a}	44.50± 3.88 ^{d}	56.83± 6.88 ^{c}

Values are mean ± SD of 6 animals per experimental group. Means within the same row carrying different superscripts were significantly different at ($P < 0.05$).